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Fatal Septicemia and Mastitis in Wild Sunda Colugos (Sunda Flying Lemurs; *Galeopterus variegates*) in Singapore

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ABSTRACT: Two free-ranging female Sunda colugos (Sunda flying lemur; *Galeopterus variegates*) were found dead in Singapore in November 2018 and February 2019. Septicemia and mastitis were diagnosed in both animals on postmortem examination. Infectious diseases have not previously been established as a cause of death in wild Sunda colugos.

Sunda colugos (Sunda flying lemurs; *Galeopterus variegates*) are one of two species in the order Dermoptera. Their exact geographical range is unknown, but they have been reported in all of Southeast Asia except the Philippines (Nasir 2013). The species is classified as Least Concern by the International Union for Conservation of Nature, but the population is decreasing because of habitat loss (Boeadi and Steinmetz 2008). In Singapore, they survive in fragmented habitats (Lim 2007). There is no information on infectious causes of mortality in wild Sunda colugos, and the animals are difficult to keep in captivity (Lim 2007). Tsuji et al. (2018) reported a mass mortality event in Indonesia, but no necropsies were performed to determine possible causes. In Singapore, vehicular trauma and predation have been recorded as causes of mortality (Lim 2007, 2014). This report on mastitis and septicemia in Sunda colugo is important to document disease occurrence in this species.

Two free-ranging adult female Sunda colugos were found dead in Mandai, Singapore, in November 2018 and February 2019. The first specimen (case 1), weighing 1.25 kg, was in rigor mortis and well preserved, and carried a live unweaned male offspring. The altricial offspring, which was furred with both eyes opened, was removed and rehabilitated, and the carcass of the adult female was examined. The second specimen (case 2), weighing 800 g, was fresh and not in rigor mortis when discovered (Fig. 1A).

Colugos have two axillary mammary glands on each side of the body. In both animals, the right mammary glands were markedly swollen with dark red discoloration, whereas the left were small with no milk expressible. Both animals were severely dehydrated. In case 1 there was a small volume of clear, blood-tinged effusion in the left thorax. The lungs from both animals were diffusely wet, heavy, mottled dark red, and foamy and had multifocal soft yellow foci measuring 2–5 mm scattered throughout the left lung parenchyma (Fig. 1B). The liver lobes in both cases were swollen and edematous, and in case 2 there were multifocal pinpoint yellow lesions scattered randomly throughout the hepatic parenchyma. In case 1, the left uterine horn was distended and well vascularized, containing an embryo measuring 10×11 mm. Samples from the heart, lungs, liver, thyroid, adrenal glands, kidneys, spleen, stomach, intestine and pancreas and the entire reproductive tract were collected for routine histologic processing.

In both cases, microscopic examination of H&E-stained sections (Fig. 1C–E) showed extensive necrotizing pyogranulomatous pneumonia with intralesional bacterial colonies. The right mammary ductular, alveolar, and surrounding connective tissues were severely necrotic with marked numbers of intact and degenerate neutrophils and bacterial colonies. The liver sections had centrilobular to diffuse and bridging degeneration of hepatocytes with multifocal random foci of necrosis. Gram staining of the lung and mammary sections from case 1 showed a mix of gram-positive and -negative cocci and rod

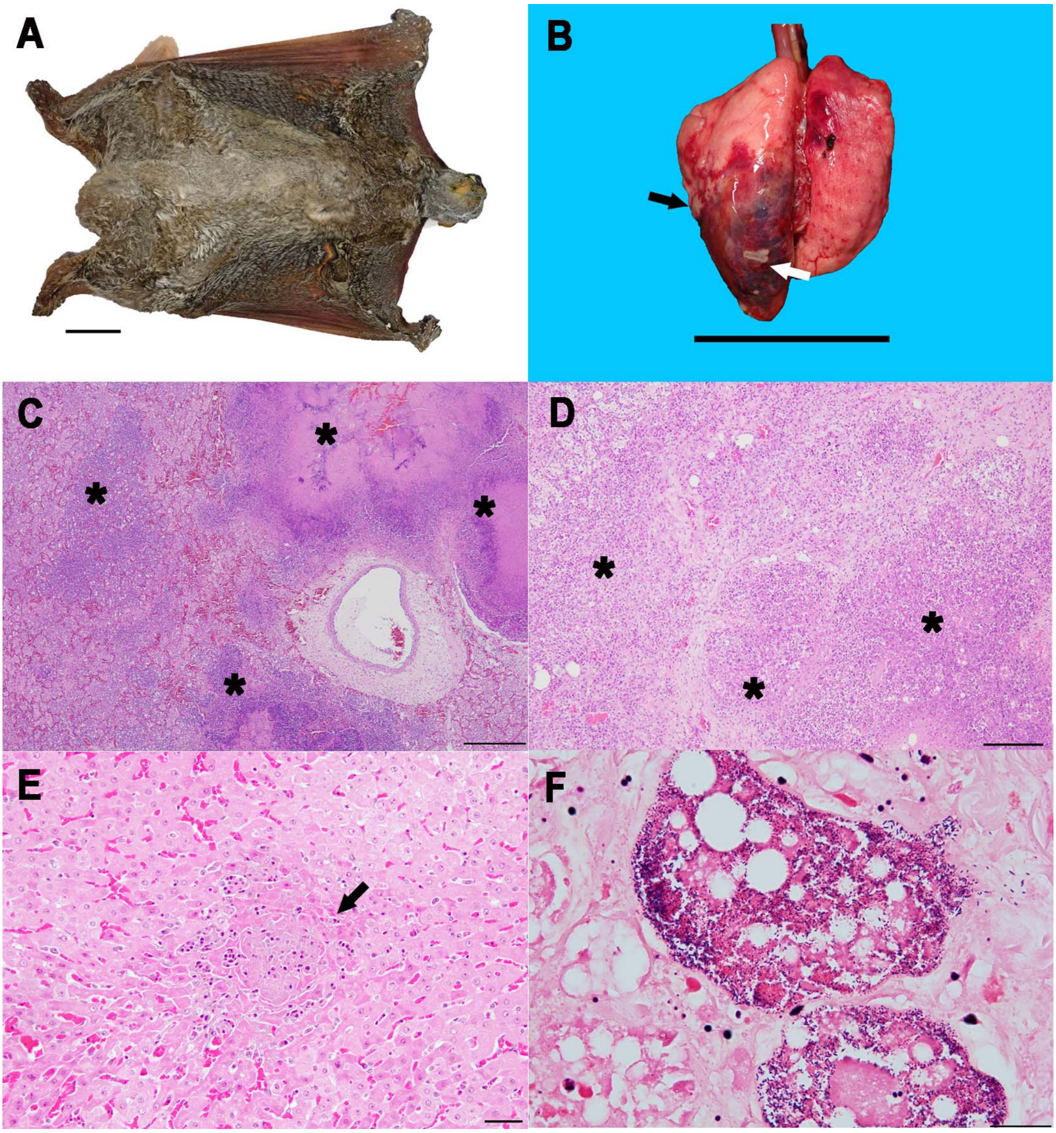

FIGURE 1. Gross photographs and photomicrographs of two wild Sunda colugos (Sunda flying lemur; *Galeopterus variegates*) with mastitis and septicemia in Singapore. (A) Dorsal view of case 2 Sunda colugo carcass. Bar=5 cm. (B) A few soft yellow foci measuring 2–5 mm scattered in the left lung parenchyma (arrows) in the lungs of case 2. Bar=5 cm. (C) Coalescing extensive necrotizing pyogranulomatous pneumonia (*) in the lungs of case 2. H&E stain. Bar=500 µm. (D) Large numbers of neutrophils have infiltrated the mammary ductular and alveolar tissue (*) in case 2. H&E stain. Bar=100 µm. (E) Foci of hepatic necrosis in the liver of case 1 (arrow). H&E stain. Bar=200 µm. (F) Large numbers of gram-positive and -negative cocci and rod bacteria have colonized the necrotic mammary alveoli of case 1. Gram stain. Bar=10 µm.

bacteria (Fig. 1F), and gram-negative bacteria were found in case 2 lung sections.

Swabs were collected aseptically from mammary tissue and lungs from both cases and inoculated on MacConkey agar and tryptic soy agar plates with 5% sheep blood at 37 C. Primary colonies were subcultured and identified using biochemical tests (API strips, bioMérieux, Marcy-l'Etoile, France). *Staphylococcus xylosus*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa* were isolated from the mammary tissue, pleural effusion, and lung, respectively, in case 1. In case 2, *Escherichia coli* was isolated from the mammary gland and lung, and *P. aeruginosa* was isolated from the liver.

Staphylococcus xylosus and *E. coli* are known to commonly cause subclinical and clinical mastitis in dairy cows (Bochniarz et al. 2014). In one study, *E. coli* caused septicemia in 22.8% of dairy cows with coliform mastitis (Wenz et al. 2006). We do not know if these pathogens in our colugos were opportunistic or primary, but it is possible that the bacterial mastitis caused by *S. xylosus* and *E. coli* in the respective cases was the primary disease that eventually resulted in septicemia and death.

Burkholderia cepacia is a ubiquitous gram-negative organism commonly encountered in soil, vegetation, and water, but rarely causes infection in immunocompetent hosts (Belchis et al. 2000); its isolation could represent postmortem colonization. However, *P. aeruginosa* is a well-documented disease-causing pathogen in many domestic species, as well as in zoo and wildlife species, causing mastitis, pneumonia, air sacculitis, enteritis, abscesses, and septicemia (Narayanan 2013; Terio et al. 2018).

The Wildlife Healthcare and Research Centre of Wildlife Reserve Singapore received 71 wild Sunda colugos from staff and the public from 2012 to 2018. Out of these, 40 were presented dead, were euthanized because of medical grounds, or died prior to release. Vehicular trauma or severe wounds were the most common findings for the dead and euthanized animals.

Unfortunately, it is not possible to determine where the two colugos that we necropsied may have acquired the infections and whether environmental factors played a contributing role. Changes in rainfall patterns and temperatures have been suggested to be potential factors in causing mortalities (Tsuji et al. 2018). Climate and rainfall data and trends of Singapore in the last 25 yr have been increasingly unusual (Meteorological Service Singapore 2019) and may be a potential factor. Habitat loss and isolated habitat fragments may also be a potential factor for disease emergence (Wilkinson et al. 2018) by altering host resistance. Singapore has undergone extensive deforestation and has only isolated pockets of natural habitats remaining (Brook et al. 2003; Agoramoorthy et al. 2006). Altered habitats can result in stress, poor nutritional status, and immunosuppression due to overcrowding; altered host–parasite dynamics; and reduced genetic resilience (Daszak et al. 2001; Lafferty and Gerber 2002). Habitat fragmentation can result in negative physiological changes in wild animals associated with stress (Martínez-Mota et al. 2007; Johnstone et al. 2012) and may subsequently predispose these animals to infections. As Singapore undergoes further development, more data on wildlife morbidities and mortalities can guide policies on land clearance and wildlife management.

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